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Customer No.: 29311

Docket No: 2427/1G772-US1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Erich HOFFMANN

Serial No.: 09/844,517

Confirmation No.: 9063

Filed: April 27, 2001

For: DNA TRANSFECTION SYSTEM FOR THE GENERATION OF INFECTIOUS INFLUENZA VIRUS

RESPONSE TO THE NOTICE TO FILE CORRECTED APPLICATION PAPERS, SUBMISSION OF SEQUENCE LISTING AND STATEMENT PURSUANT TO 37 C.F.R. § 1.821

January 9, 2002

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

In response to the Notice to File Corrected Application Papers dated December 18, 2001, enclosed please find (i) a substitute specification in compliance with 37 C.F.R. §1.52 (attached

as Exhibit A) and (ii) a computer readable form (diskette) and a paper copy containing the Sequence Listing pursuant to the requirements of 37 C.F.R. §1.821-1.825 (attached as Exhibit B).

Please enter the enclosed documents in the application file.

REMARKS

As stated in the Notice to File Corrected Application Papers received in the above-identified patent application (copy attached as Exhibit C), applicants should submit in compliance with 37 C.F.R. §1.52 a substitute specification containing the proper margins. Such substitute specification is attached as Exhibit A. Applicants note that, compared to the application as filed, this substitute specification contains a number of formal amendments. Thus, a customer number on page 1 has been corrected to the number 29311 in accordance with submission of October 2, 2001. Also, some of the SEQ ID NOS (at pages 56-58 of the substitute specification) have been changed to correct the use of two different SEQ ID NOS for a single sequence. SEQ ID NOS in the substitute specification correspond to the SEQ ID NOS provided in the Sequence Listing (attached as Exhibit B). Three pages showing the differences between the substitute specification and the original specification (generated by CompareRite™) are attached as Exhibit D. In these pages, deletions appear as strikethrough text surrounded by {} and additions appear as bold text surrounded by []. No new subject matter has been added as a result of these amendments.

As further stated in the Notice to File Corrected Application Papers, the present application fails to comply with the sequence disclosure requirements set forth in 37 C.F.R. §1.821

through §1.825. Applicants are therefore requested to provide an initial Sequence Listing both as paper copy and as a computer readable form.

Pursuant to the requirements of 37 C.F.R. §1.821 through §1.825 for Sequence Listings, a computer readable form (diskette) and a paper copy containing the Sequence Listing are enclosed. Applicants note that the sequences contained in the enclosed Sequence Listing are disclosed, for example, at page 48, lines 13-18; page 54, line 7 - page 55, line 7, and page 56, lines 1-4 of the specification as filed.

STATEMENT PURSUANT TO 37 C.F.R. § 1.821

Enclosed herewith is a computer readable form (diskette) and a paper copy containing sequence disclosure as requested by the Office. A copy of the Notice to File Corrected Application Papers is also enclosed. Pursuant to Rule 821, applicants herein state that the contents of the attached paper entitled "SEQUENCE LISTING" and of the accompanying identically labeled diskette, specifically the ASCII-encoded file therein labeled "seqlist.txt", are identical and that the submission contains no new matter.

CONCLUSION

Applicants request entry of the foregoing amendments and remarks in the file history of this application. Applicants respectfully submit that the application is now in compliance with the Application Requirements pursuant to 37 C.F.R. §1.52 and the Sequence Listing Requirements

pursuant to 37 C.F.R. §1.821 et seq. Substantive examination of the application is respectfully requested and a favorable determination respectfully solicited.

Respectfully submitted,

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pHW2000. The eight plasmids containing the cDNA of A/Teal/HK/W312/97 (H6N1) (pHW241-PB2, pHW242-PB1, pHW243-PA, pHW244-HA, pHW245-NP, pHW246-NA, pHW247-M, and pHW248-NS) were constructed by reverse-transcriptase polymerase chain reaction (RT-PCR) amplification of the viral RNA. The primers used in the PCR reaction contained segment-specific sequences at their 3' end and *Bsm*BI or *Bsa*I restriction site sequences at their 5' end. After digestion of the PCR products with *Bsm*BI or *Bsa*I, the fragments were cloned into the vector pHW2000 (Figure 3A). The sequences of the primers used for amplification of the genome of A/teal/HK/W312/97 (H6N1) follow. The primers are shown from left to right corresponding to the 5' and 3' ends. The influenza A specific nucleotides are underlined.

· NS:

Bm-NS#1: TATTCGTCTCAGGGAGCAAAAGCAGGGTG (SEQ ID NO:5)

Bm-NS#2: ATATCGTCTCGTATTAGTAGAAACAAGGGTGTTT (SEQ ID NO:6)

· M:

Bm-M#1: TATTCGTCTCAGGGAGCAAAAGCAGGTAG (SEQ ID NO:7)

Bm-M#2: ATATCGTCTCGTATTAGTAGAAACAAGGTAGTTTTTT (SEQ ID NO:8)

· NA:

Bm-NA1-1: TATTCGTCTCAGGGAGCAAAAGCAGGAGTTTAACATG (SEQ ID NO:9)

Bm-NA-1413R: ATATCGTCTCGTATTAGTAGAAACAAGGAGTTTTT (SEQ ID NO:10)

· HA:

Bm-H6-1: TATTCGTCTCAGGGAGCAAAAGCAGGGGAAAATG (SEQ ID NO:11)

Bm-NS#2: ATATCGTCTCGTATTAGTAGAAACAAGGGTGTTT (SEQ ID NO: ~~{12)}~~ **[6)]**

(note:HA and NS segment have the identical sequence in this part of the noncoding region)

· NP:

Ba-NP-1: TATTGGTCTCAGGGAGCGAAAGCAGGGTA (SEQ ID NO: ~~{13)}~~ **[12)]**

Ba-NP1565R: ATATGGTCTCGTATTAGTAGAAACAAGGGTATT (SEQ ID NO: ~~{14)}~~ **[13)]**

· PA:

Bm-PA1-1: TATTCGTCTCAGGGAGCGAAAGCAGGTACTGATCC (SEC ID NO: ~~{15)}~~ **[14)]**

Bm-PA1-2231R: ATATCGTCTCGTATTAGTAGAAACAAGGTACTTTTT (SEC ID NO: ~~{16)}~~ **[15)]**

· PB1:

Bm-PB1a-1: TATTCGTCTCAGGGAGCGAAAGCAGGCAAACC (SEC ID NO: ~~{17)}~~ **[16)]**

Bm-PB1-2341R: ATATCGTCTCGTATTAGTAGAAACAAGGCATTT (SEC ID NO: ~~{18)}~~ **[4)]**

· PB2:

Ba-PB2-1: TATTGGTCTCAGGGAGCGAAAGCAGGTCAATTATATTC (SEC ID NO: ~~{19)}~~ **[17)]**

Ba-PB2-2341R : ATATGGTCTCGTATTAGTAGAAACAAGGTCGTTTTT (SEC ID NO: ~~{20)}~~ **[18)]**

The RT-reaction was performed with the primer 5'-AGCAAAAGCAGG-3' (SEC ID NO: ~~{21)}~~ **[19).]**

To ensure that the viral cDNAs derived from RT-PCR amplification in the expression plasmids did not have unwanted mutations, the inserted cDNAs were sequenced.

Viruses and cell culture. Influenza viruses A/WSN/33 (H1N1) and A/Teal/HK/W312/97 (H6N1) were propagated in 10-day-old eggs. Madin-Darby canine kidney (MDCK) cells were maintained in modified Eagle Medium (MEM) containing 10% FBS. 293T human embryonic kidney cells were cultured in Opti-MEM I (Life Technologies, Gaithersburg, Maryland) containing 5% FBS. For the transfection experiments six well tissue culture plates were used. The day before transfection confluent 293T and MDCK cells in a 75 cm^2 flask were trypsinized and 10% of each cell line was mixed in 18 ml OptiMEM I; 3 ml of this cell suspension was seeded into one well of a six well plate. The cocultured MDCK and 293T cells (0.2 - 1 x 10^6 cells per well each) were used for the transfection experiments. TransIT LT-1 (Panvera, Madison, Wisconsin) was used according to the manufacturer's

instructions to transfect the cells. Briefly, 2 µl of TransIT LT-1 per 1 µg of DNA was mixed, incubated at room temperature for 45 min, and added to the cells. Six hours later, the DNA-transfection mixture was replaced by Opti-MEM I. Thirty hours after transfection, 1 ml of Opti-MEM I containing TPCK-trypsin was added to the cells; this addition resulted in a final concentration of TPCK-trypsin of 0.5 µg/ml in the cell supernatant. The virus titer of the cell supernatant was determined by titration of the supernatant on MDCK cells.

RNA isolation and RT-PCR. Viral RNA was isolated from virus particles with the RNeasy-Kit (Qiagen, Valencia, California), which was used according to the manufacturer's instructions. For characterization of recombinant influenza viruses, the Access RT-PCR kit (Promega, Madison, Wisconsin) was used according to the protocol provided. The following primers were used in the RT-PCR experiments: Bm-NS#1 (5'-TAT TCG TCT CAG GGA GCA AAA GCA GGG TG-3; SEQ ID NO:5) and Bm-NS#2 (5'-ATA TCG TCT CGT ATT AGT AGA AAC AAG GGT GTT TT-3; SEQ ID NO: ~~(12))~~ **[6)]**. RT-PCR experiments were performed by using the PTC-200 DNA engine (MJ Research, Watertown, Massachusetts). The amplification program started with 1 cycle at 48°C for 45 min and 1 cycle at 94°C for 2 min. These cycles were followed by 40 cycles at 94°C for 20 sec, 52°C for 30 sec, and 72°C for 40 sec; the program ended with one cycle at 72°C for 5 min. The PCR products were analyzed by agarose gel electrophoresis and sequenced with the primer Bm-NS#1. The Center for Biotechnology at St. Jude Children's Research Hospital determined the sequence of template DNA by using rhodamine or dRhodamine dye-terminator cycle sequencing ready reaction kits with AmpliTaq® DNA polymerase FS (Perkin-Elmer, Applied Biosystems, Inc. [PE/ABI], Foster City, CA) and synthetic oligonucleotides. Samples were subjected to electrophoresis, detection, and analysis on PE/ABI model 373, model 373 Stretch, or model 377 DNA sequencers.

Results

Establishment of the pol I - pol II system for the generation of A/WSN/33 (H1N1). Because the genome of influenza A virus contains eight segments, it was reasoned that the insertion of all eight influenza A cDNAs between a pol I promoter and a pol II promoter should result in the transcription of the eight vRNAs, all viral in RNAs, and in the